Metagenomic Fingerprinting of Rainwater Harvesting Systems

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BACKGROUND

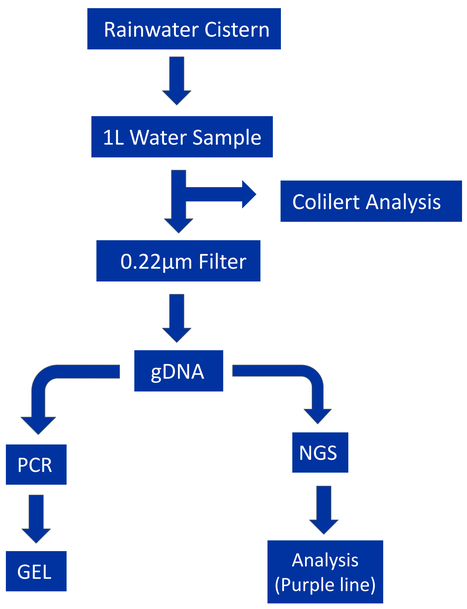
- Rainwater collection systems are used to collect and store rainwater from the environment (figure 1).
- These collection systems tend to accumulates containments like fecal matter, insect larvae, bacteria, and pathogens, which could lead to contamination and possible health hazards.
- Colilert® and 16s rRNA sequencing were used to detect bacteria present in systems.
- A Colilert® detection system uses MPN to estimate coliform bacteria populations present in water[1,2].
- The 16s rRNA gene was used to identify bacteria using metagenomic sequencing.
- "Metagenomic Fingerprinting" provided an overview of the diversity of bacteria in the rainwater collection systems[3].
- Metagenomic analysis has rarely been used on samples of rainwater collection systems; however, it is an ideal method of determining the microbial diversity in these systems.

RESEARCH QUESTION & HYPOTHESIS

Research Question: What is the microbial diversity in rainwater harvesting systems and what factors affect that diversity?

Hypothesis: Application of metagenomic fingerprinting will demonstrate changes in the diversity of bacteria based on environmental factors.

METHODS AND MATERIALS



Primers:
- 16S_515F: GTGYCAGCMGCCGCGGTAA
- 16S_806R: GGACTACNVGGGTWTCTAA

RESULTS

Figure 1. Typical example of a Rainwater Harvesting System (Source HB).

Source (Replicates of 3)	pH	Tank Volume	Water Temperature (° C)
FS26	5.93	5678	7.3
FS8L	7.54	4542	9.7
HB	7.66	15141	6.9
CDP	7.98	1155	5.2
CF1	7.92	2082	13.3

Table 1. Metadata for water samples.

Sample Site	Average Coliform Concentration (MPN/100ml)
FS8L	>2419.6
HB	19.67
FS26	25.27

Table 2. Average coliform presence by Colilert®. Only sites shown had coliform present. Remaining samples detected no coliform. Samples were performed in triplicates.

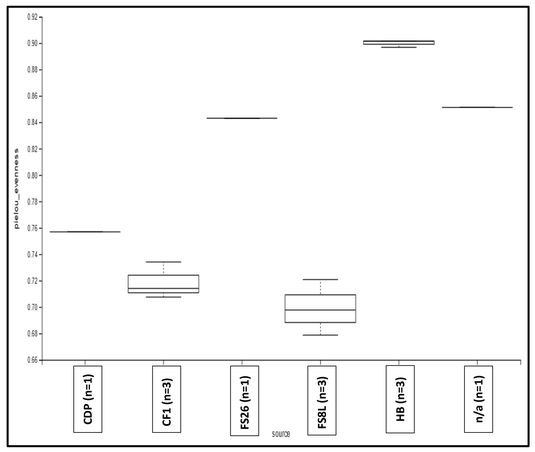


Figure 2. Alpha Diversity using Pielou's Evenness. Alpha diversity is highly variable between sources.

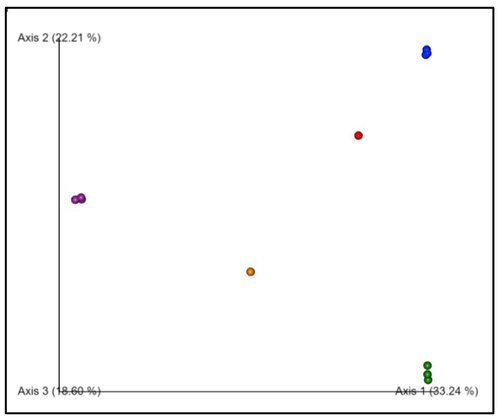


Figure 3. Beta Diversity using Bray Curtis Distance. (Purple: HB , Orange:FS26, Red: CDP, Blue:CF1, Green:FS8L)

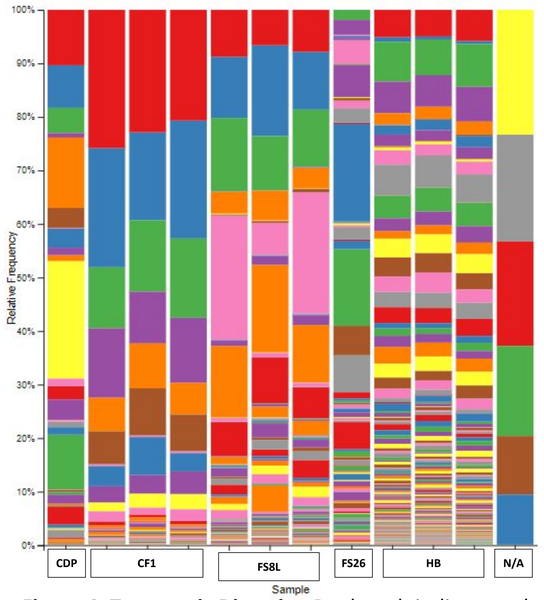


Figure 4. Taxonomic Diversity. Bar length indicates relative frequency of bacteria present.

	OTU (Operational Taxonomic Unit)		FS8L	HB	CDP	CF1	FS26
	Family	Genus					
Most Frequent	1. Oxalobacteraceae	Polynucleobacter	X	—	X	X	—
	2. Comamonadaceae	—	X	X	X	X	—
	3. Chitinophagaceae	—	X	X	X	X	—
	4. Rhodospirillaceae	—	—	X	—	—	X
	5. Sphingomonadaceae	Novosphingobium	X	—	X	X	—
Pathogenic	1. Legionellaceae	—	X	X	X	X	X
	2. Enterobacteriaceae	—	X	—	—	—	—
	3. Clostridiaceae	Clostridium	X	X	—	—	—
	4. Bacillaceae	Bacillus	X	X	—	—	—

Table 3. Presence or absence of OTU's in each source organized by frequency and recognized pathogens.

CONCLUSIONS

- Colilert® detected the presence of coliform bacteria in some barrels
- Metagenomics Fingerprinting provides a robust indication of microbial diversity in rainwater sources
  - Results show differences between each sources
  - Alpha diversity indicates diversity within sources
  - Beta diversity indicates diversity between sources
  - Taxonomic diversity indicates a large amount of diversity for each source
- **Limitations**
  - Using pre packaged version of QIIME limited analysis and background information
  - 300 bp sequences of 16s rRNA were insufficient to identify OTU's to species.
  - Preliminary data includes only one time point

FUTURE DIRECTIONS

- This preliminary data will be used in another study using EMA qPCR to quantify bacteria.
- A second analysis of a late summer sample will be conducted and compared for seasonal variability.
- We plan to continue sampling so we can improve statistical analysis.

ACKNOWLEDGMENTS

- The Longwood Office of Student Research provided funding.
- Dr. Ray Enke, of James Madison University, coordinated the metagenomic sequencing.
- Metagenomic Fingerprint analysis was completed using Cyverse DNA Subway

REFERENCES

1. EPA. 2003. Guidelines Establishing Test Procedures for the Analysis of Pollutants; Analytical Methods for Biological Pollutants in Ambient Water, Federal Register. 68(139): 43272-43283.
2. EPA. 2018. Expedited Approval of Alternative Test Procedures for the Analysis of Contaminants in Under the Safe Drinking Water Act; Analysis and Sampling Procedures. Federal Register. 83(198):51636-51652.
3. Alindonosi AR, Baeshen MN, Elsharawy NT. 2021. Prospects For Diatoms Identification Using Metagenomics: A Review. Applied Ecology and Environmental Research. 19(6):4281–4298. doi:10.15666/aeer/1906_42814298